Section II (Remarks)

A. Regarding the Amendments

Claim 1 has been amended as set forth in the above Complete Listing of the Claims. No new matter has been added, as defined by 35 U.S.C. § 132.

As amended, the claims are supported by the specification and the original claims. Specifically, support for the amendment of claim 1 is provided in the specification as originally filed at page 3, lines 3-14, page 7, lines 25-28 and the Examples.

Claims 1, 2 and 4-13 remain pending and under examination.

In view of the finality of the November 10, 2010 Office Action and to ensure substantive consideration of this response, a Request for Continued Examination is concurrently submitted herewith, together with payment of the appertaining RCE fees (see *infra*, "Conclusion").

B. Rejection Under 35 U.S.C. § 102

In the Office Action mailed November 10, 2010, the examiner maintained the rejection of claims 1, 6 and 12 under 35 U.S.C. §102(b) as being anticipated by European Patent Application No. EP 0702081 (hereinafter "Morota et al.") Applicants respectfully traverse the rejection.

It is well established that anticipation of a claim requires the disclosure in a single prior art reference of each element of the claim under consideration. (*Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987.) Morota et al. do not anticipate all elements of each of claims 1, 6 and 12. Accordingly, claims 1, 6 and 12 are not anticipated by Morota et al.

In the response submitted August 2, 2010, applicants demonstrated that the method of Morota et al. differs from the claimed methods in that the method of claim 1 recites "organized, differentiated, polarized and functionally active two-dimensional cell cultures," where the cells are in a "suitable functional state," further recites a specific combination of gelatin concentration, solidification temperature, storage temperature and time of storage, and still further recites that the gelatin coats the two-dimensional cell culture.

In response to applicants' arguments, with regard to the conditions of the method, the examiner alleged that "Morota et al clearly describe the claimed method wherein gelatin is applied at about 5% and solidifying takes place at 20 deg. Cent. for a period of 3 to 5 days of which 96 hours is equivalent of 4 days." (Final Office Action, p. 3.) With regard to the structure of the cells, the examiner responded that "[t]he double layer structure of dermis cells will inherently have the characteristics of differentiated, polarized and functionally active two-dimensional cell cultures." (Final Office Action, p. 4.) Applicants strenuously disagree with the examiner's assumptions and the resulting conclusion that the method of Morota et al. anticipates independent claim 1.

With regard to the three-dimensional cell cultures described by Morota et al., it is the examiner's position that "...three-dimensional cell layers require a series of two-dimensional cell layers..." In support of such statement, the examiner cited columns 2-3 of Morota et al. Applicants respectfully direct the examiner's attention to Morota et al. at col. 1, lines 56-57 and col. 2, lines 8-9. It is a stated objective of Morota et al. to form a "desired three-dimensional structure..." (emphasis added.)

The method of Morota et al. produces cultured skin with a two-layered structure generated from a first sponge and a second sponge. At col. 3, lines 25 the three-dimensional structure is described where a "first cell is dropped into first sponge and proliferate [sic] three-dimensionally within said porous structure..." The examiner's assertion that "[t]he double layer structure of dermis cells will inherently have the characteristics of differentiated, polarized and functionally active two-dimensional cell cultures" is unsupported by the disclosure of Morota et al. Nowhere in Morota et al. are two-dimensional cell cultures described. Nor is it apparent where the examiner finds support for the conclusion that double layer structures inherently have the same characteristics as two-dimensional cell cultures.

The examiner's attention is respectfully directed to the Declaration of Francesc Mitjans Under 37 CFR §1.132, submitted herewith. Dr. Mitjans, an independent scientist, has reviewed the application and the rejections raised by the examiner and has provided his independent conclusion, as stated in the Declaration, that the *in vitro* and polarized cell model recited in applicants' claims is innovative, in that applicants devised a way to immobilize the two-dimensional cell cultures on an asymmetric support to enable induction of differentiation and polarization and subsequent coating with gelatin. The methods of Morota et al. do not provide such means for two-dimensional cell cultures.

Furthermore, Morota et al. does not anticipate the conditions of applicants' claimed method. The examiner's attention is respectfully directed to claim 1, parts (a)-(c), where the method specifically includes the following characteristics: a gelatin concentration of 1-5%, solidifying the gelatin at 15-25°C, and storing and/or transporting at 15-25°C for a period of up to 96 hours. Morota et al. fails to describe a storage/transporting step, as in applicants' claimed invention.

Furthermore, claim 1, part (a) provides the step of "**coating** an organized, differentiated, polarized and functionally active two-dimensional cell culture…" Morota et al. fails to describe a coating step. In Morota et al., the gelatin solution does not coat the cultured tissue, but the tissue is simply immersed in "solated gelatine," which is then gelatinized, in order to ensure that the tissue is not moved or separated. As such, the gelatin simply surrounds the sponge containing the dermal layer of the tissue (see page 4, col. 5, line 15), so that epidermis is in contact with the atmosphere and dermis is in contact with a nutrient supply. The requirement of contacting epidermis with the atmosphere while dermis is partially immersed in gelatin solution is also noted in examples 1 and 2 (respectively, page 5, col. 7, lines 13-16 and col. 8, lines 10-12) as well as figure 1 of Morota et al., reproduced below:

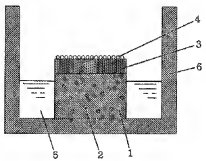

Figure 1 illustrates that the medium **5** surrounds the first layer of tight-crosslinked collagen sponge **1** and human fibroblast **2**, to provide nutrients, but that the second layer of loose-crosslinked collagen sponge **3** and human keratinocyte **4** is in contact with the atmosphere. This application of the gelatin is stated even more clearly in Example 2 of Morota et al., at col. 8, lines 6-12:

> "The skin model obtained in example 1(3) having double-layer structure of dermis and epidermis was placed at the center of a 6-well plate. The gelatin sol (3 ml) prepared in example 2(1) was added around the model. When using this liquid volume, **surface epidermis layer was contacted with gas phase**, i.e. air, and **dermis layer was then immersed in gelatin solution**…" (emphasis added)

The terms "coating," as used in applicants' claims, and "surrounding"/"immersing" as used in Morota et al., are not interchangeable or homologous and consequently, Morota et al. do not describe a "method of storing and/or transporting *in vitro* organized, differentiated, polarized and functionally active two-dimensional cell cultures…[comprising in part] …coating an organized, differentiated, polarized and functionally active two-dimensional cell culture…" as is recited in claim 1.

The examiner's attention is again respectfully directed to Dr. Mitjans' Declaration, submitted herewith. Dr. Mitjans declares that the methods of applicants' invention provide a direct coating of the whole cellular model, leaving no cells in direct air contact. Dr. Mitjans further declares that "[t]here is nothing in the prior art similar to this fact because of the usual death of cells, as soon as their whole surface is in contact with this kind of medium. The uniqueness of the claimed methodology solves this last cellular death problem." This is corroborated by the statements in Morota et al. that the epidermis needs to be in contact with the atmosphere, to prevent problems of swelling and cell death (*see*, Morota et al., col. 5, lines 29-30.) Therefore, a teaching that a portion of a two layer culture must remain in contact with the environment would, as noted by Dr. Mitjans, "lead away a skilled person from completely coating with gelatine any cell culture, since this could seriously affect the cell functionality." (Dr. Mitjans Declaration, page 2.)

The examiner further stated that the cells of Morota et al. do maintain functionality, as described at col. 8, lines 29-34. (Final Office Action, p. 8.) However, such tissue slice is not a slice of a culture fully immersed or coated with gelatin. Additionally, Morota et al. simply visually observe the resulting viability of the cells, and do not examine the functionality of the cells. Therefore, no direct comparison of functionality can be made. One of skill in the art would expect, as expressly stated by Morota et al., that an epidermal layer that is not permitted to remain in contact with the environment would lead to cell death within that layer.

Still further, Morota et al. fails to describe an asymmetric support on which the culture is immobilized.

Accordingly, Morota et al fails to anticipate the following claimed elements of applicants' invention, as recited in independent claim 1:

- coating a two-dimensional cell culture;
- coating a two-dimensional cell culture immobilized on an asymmetrical

support;

- coating a cell culture comprising cells in suitable functional state prior to coating; and
- storing/transporting the coated culture where the cells are maintained in the suitable functional state.

As such, claim 1 is not anticipated by Morota et al. Claims 6 and 12 are patentable for the same reasons advanced above in support of the patentability of claim 1.

Since Morota et al. does not describe a method as set forth in claims 1, 6 and 12, Morota et al. does not anticipate the claimed invention. Accordingly, withdrawal of the rejection of claims 1, 6 and 12 under 35 U.S.C. § 102(b) as being anticipated by Morota et al., is respectfully requested.

C. Rejection Under 35 U.S.C. § 103

In the Office Action mailed November 10, 2010, the examiner maintained the rejection of claims 1 to 13 under 35 U.S.C. §103(a) as being unpatentable over European Patent Application No. EP 1127580 (hereinafter "Curatolo et al.") and Morota et al. or over International Patent Application Publication No. WO 01/66783 (hereinafter "Lee et al.") in view of Morota et al. Initially, it is noted that claim 3 was cancelled in the Response filed August 2, 2010 and the rejections are therefore addressed herein as applicable to pending claims 1, 2 and 4-13. Applicants traverse the rejections of claims 1, 2 and 4-13.

In the response submitted August 2, 2010, applicants demonstrated that both of the cited combinations of Curatolo et al. in view of Morota et al. or Lee et al. in view of Morota et al. fail to describe all elements of applicants' claimed invention. As such, neither of the cited combinations of references renders claim 1 obvious, or any of claims 2 and 4-13, dependent therefrom.

As the Examiner acknowledged on page 5 of the Final Office Action, neither of the cited primary references Curatolo et al. nor Lee et al. expressly teach a method of storing by coating the cell culture support with gelatin, solidifying the gelatin, storing the cells, or liquefying the gelatin. The passages of Curatolo et al. and Lee et al. indicated by the examiner merely describe cell

culture techniques as *in vitro* models for assessing the azithromycin transport across Caco-2 Cell Monolayers (Curatolo et al.) or the inhibitory activity against VEFG-Induced HUVEC proliferation (Lee et al.). Both of these references are related to the growth of cells and subsequent analysis, but neither reference, in any way discloses or suggests a method that includes coating the cell culture support with gelatin.

As discussed in detail above, Morota et al. describes a cultured tissue immersed in gelatin, but not coated with gelatin. Morota et al. teaches away from complete immersion of the cultured tissue, as such immersion would be expected to cause cell death of the epidermal layer. As such, Morota et al. does not remedy the deficiencies of either of Curatolo et al. or Lee et al.

Additionally, neither of cited primary references Curatolo et al. or Lee et al., describe a method of storing and/or transporting cell cultures so that the cell culture maintains its functional state. Nor does either of the cited primary references, Curatolo et al. or Lee et al., describe the specific steps included in the method recited in independent claim 1. In the Response mailed August 2, 2010, applicants demonstrated that Morota et al. does not cure the deficiencies of either of Curatolo et al. or Lee et al. Specifically, neither primary reference in combination with Morota et al., provides a method of storing and/or transporting a two-dimensional cell culture where, in relevant steps, the culture is coated with gelatin and maintains its suitable functional state through storage and/or transportation.

It is a stated objective of applicants' invention to provide "ready-to-use" two-dimensional cell cultures. An essential aspect of the ready-to-use state is that the cultures be of "suitable functional state" prior to coating with gelatin and that the cultures maintain such functionality through storage and/or transport. In order to emphasize these aspects of the invention, the phrase "prior to coating" has been added to claim 1, part (a), and the phrase "comprising cells in said suitable functional state" has been added to claim 1, part (c). The term "suitable functional state" is defined in the application at page 7, lines 25-28:

> "In the context of the present invention, *suitable functional state* is understood to be the state the viable cell cultures present when they are capable of performing the function they have been assigned in the assay."

Applicants' claimed invention provides a solution to the problem of maintaining the functional state of the specific cell cultures defined in claim 1, so that they may be used in an assay within a sufficient timeframe following storage and/or transport. By contrast, Morota et al. does not

relate to *in vitro* models, nor to polarized cells, and does not suggest the surprising result that organized, differentiated polarized two-dimensional cells cultures could be stored and transported by the methods of applicants' claimed invention and that the cultures will maintain their functionality for *in vitro* tests after storage, as shown in the examples.

The methods recited in applicants' claimed invention unexpectedly make the maintenance of the physiological properties of two-dimensional cell cultures possible, in addition to the protection of mechanical properties during the storing and transport of cell cultures. This allows use of the culture, and its functional properties once the gelatin is removed, up to 9 days thereafter (*see* Specification, example 1, p. 12, lines 1-11). Indeed, the fixing method defined in claim 1 allows the immobilization of a Caco-2 cell culture for up at 4 days at room temperature without affecting its functional barrier state (established by measuring Trans Epithelial Electric Resistance, TEER) and once the gelatin has been removed to perform barrier permeability assays. Additionally, it has been shown for other two-dimensional cell types that the functionality is maintained by adhesion, migration and invasion assays (*see* Specification, example 2 and last paragraph of the description).

It is understood, as noted by the examiner, that the characteristics described in the foregoing paragraph are not elements of applicants' claims, however, such characteristics are illustrative of the claimed features that the cultures will maintain their functionality through storage and/or transportation.

These advantages are particularly relevant for polarized, differentiated two-dimensional cell cultures due to their complexity and their temporal limitations, since once a functional culture is obtained, it is particularly difficult to maintain its functional properties unimpaired and to allow passage of a sufficient time for making *in vitro* assays without losing the functionality of the cell culture (*see* Specification, p. 7, lines 7-34).

As discussed in the Response submitted August 2, 2010, polarization is a term that is well known to one of skill in the art of cell cultures. The term relates to cells that exhibit structural and functional asymmetry between apical and basolateral surfaces. The cells have plasma membranes specialized to receive specific chemical signals originating from the external (or apical surface) and internal (or basolateral surface) environments of the organism. This feature makes this type of cell cultures especially complex since they require continuous manipulation to

maintain the properties that make them a suitable model to imitate the natural barriers (veins, intestine, etc.) of the organism. For example, Caco-2 cells (polarized cells) require 21 days to reach the state of differentiation which allows many of the properties of intestinal mucous to be reproduced, and their use is prolonged only during a window of approximately 3 to 5 days (*see* Specification, p. 1-2).

The difficulties overcome by applicants are further affirmed by Dr. Mitjans' Declaration, where he stated that "this kind of models [*in vitro* and polarized] are of such structural complexity, that it is almost impossible for the skilled person to maintain their functional properties intact during a certain time frame in order to perform related cellular assays." (Declaration, p. 2.) However, applicants' invention provides such models.

Both Curatolo et al. in view of Morota et al. and Lee et al. in view of Morota et al. fail to provide any derivative basis for the claimed invention. Specifically, the two cited combinations of references fail to describe a method that comprises coating of a cell culture and also fail to describe a method of storage and/or transport in which the culture maintains a suitable functional state in which it is "ready-to-use." Accordingly, no basis of *prima facie* obviousness of the claimed invention is presented by such cited references.

Additionally, there would have been no logical reason for one of skill in the art to combine such references. In looking for a solution to the problem of creating a polarized, differentiated two-dimensional cell culture and storage and transporting of the same, one of skill in the art would not have considered the teaching of Morota et al.

The fixing method described in Morota et al. is used with the aim of avoiding cellular death and mechanical impairment in a three-dimensional cultured tissue. In contrast to the examiner's assertion, Morota et al. does not analyze whether the dermal and/or epidermal layers maintain any functionality. Instead, Morota et al. simply visually observe by microscopy (*see* p. 5, col. 8, lines 19-34), to determine whether the cells maintained their structure and whether the cells survived the storage. However, in Morota et al. there is no assessment of whether the cellular model is functionally active.

Furthermore, Morota et al. does not relate to *in vitro* models, nor to polarized cells, and does not suggest the surprising result that, organized, differentiated polarized two-dimensional cell

cultures could be stored and transported by the method of applicants' claimed invention and that the cultures will maintain "ready-to-use" functionality for *in vitro* tests after storage, as shown in the examples.

Additionally, in the fixing system described in Morota et al., the tissue culture is not coated by gelatin. In Morota et al. the epidermal layer formed upon the dermal layer is not immersed in the gelatin solution (the epidermal layer is kept in contact with the atmosphere). It is only the first sponge, wherein the dermal cells are cultured, that is surrounded by the gelatin solution. This is detailed in Figure 1 of Morota et al., reproduced above. In fact, Morota et al. teaches that epidermal cells must not be immersed in the gelatin solution, in order to avoid their death. Thus, Morota et al. teaches away from a **coating** step, as recited in applicants' claimed invention.

In considering a reference for its effect on patentability, the reference is required to be considered in its entirety, including portions that teach away from the invention under consideration. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984); MPEP § 2141.02. Morota et al. teach one of skill in the art away from completely coating an organized two-dimensional cell culture with gelatin, since, according to the teaching of the Morota publication, this could detrimentally affect the cell functionality.

Furthermore, the support described in Morota et al. consists of a combination of two sponges with different physical and chemical features (p. 3, col. 4, lines 20-24), which allows, on the one hand, the three-dimensional proliferation of fibroblasts (dermis) within the structure of the first sponge, and on the other hand, the proliferation of the keratinocytes (epidermis) on the surface of the second sponge. It would not be obvious for the skilled person that the method of Morota et al. would equally work in absence of the specific sponges.

The asymmetric support recited in claim 1, part (a) of applicants' claimed invention, is an essential element to the cells' being in a suitable functional state (polarization) prior to coating with gelatin. Such utility of the asymmetric support is exemplified in example 1, part 2.

Based on the foregoing, Curatolo et al. in view of Morota et al. and Lee et al. in view of Morota et al. fail to provide any logical basis for the methods and kits recited in claims 1, 2 and 4-13.

Specifically, the cited combinations of references do not address the problems related to the preservation of functional properties during storing and transport of organized two-dimensional cell cultures, nor the possibility of maintaining the functionality of the cultures in a "ready-to-use" state. Additionally, the cited combinations of references do not include a method wherein a culture is coated with gelatin as an essential step of maintaining such functionality.

Neither Curatolo et al. in view of Morota et al., nor and Lee et al. in view of Morota et al. render the claimed invention obvious. Accordingly, withdrawal of the rejection of claims 1, 2 and 4-13 under 35 U.S.C. § 103(a) as being obvious over Curatolo et al. in view of Morota et al. and Lee et al. in view of Morota et al. is respectfully requested.

CONCLUSION

Based on the foregoing, all of applicants' pending claims 1, 2 and 4-13 are patentably distinguished over the art, and in form and condition for allowance. The examiner is requested to favorably consider the foregoing and to responsively issue a Notice of Allowance.

The time for responding to the November 10, 2010 Office Action without extension was set at three months, or February 10, 2011. Applicants hereby request a three month extension of time under 37 C.F.R. § 1.136 to extend the deadline for response to and including May 10, 2011. Payment of the extension fee of $555.00 specified in 37 C.F.R. § 1.17(a)(3) and the RCE fee of $405.00 specified in 37 C.F.R. § 1.17(e), as applicable to small entity, is being made by on-line credit card authorization at the time of EFS submission of this Response. Should any additional fees be required or an overpayment of fees made, please debit or credit our Deposit Account No. 08-3284, as necessary.

If any issues require further resolution, the examiner is requested to contact the undersigned attorneys at (919) 419-9350 to discuss same.

Respectfully submitted,

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Enclosures:
Declaration Under 37 CFR §1.132

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